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ENDOTOXIN EXPOSURE AND INFLAMMATION MARKERS AMONG AGRICULTURAL WORKERS IN COLORADO AND NEBRASKA

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The adverse respiratory effects of agricultural dust inhalation are mediated in part by endotoxin, a constituent of gram-negative bacterial cell walls. This study quantified personal work-shift exposures to inhalable dust, endotoxin, and its reactive 3-hydroxy fatty acid (3-OHFA) constituents among workers in grain elevators, cattle feedlots, dairies, and on corn farms. Exposures were compared with post-work-shift nasal lavage fluid inflammation markers and respiratory symptoms. Breathing-zone personal air monitoring was performed over one work shift to quantify inhalable dust (Institute of Medicine samplers), endotoxin (recombinant factor C [rFC] assay), and 3-OHFA (gas chromatography/mass spectrometry). Post-shift nasal lavage fluids were assayed for polymorphonuclear neutrophils (PMN), myeloperoxidase (MPO), interleukin 8 (IL-8), albumin, and eosinophilic cation protein (ECP) concentrations. The geometric mean (GSD) of endotoxin exposure (rFC assay) among the 125 male participants was 888 ± (6.5) EU/m^3, and 93% exceeded the proposed exposure limit (50 EU/m^3). Mean PMN, MPO, albumin, and ECP levels were two- to threefold higher among workers in the upper quartile of 3-OHFA exposure compared to the lowest exposure quartile. Even numbered 3-OHFA were most strongly associated with nasal inflammation. Symptom prevalence was not elevated among exposed workers, possibly due to endotoxin tolerance or a healthy worker effect in this population. This is the first study to evaluate the relationship between endotoxin's 3-OHFA constituents in agricultural dust and nasal airway inflammation. More research is needed to characterize the extent to which these agents contribute to respiratory disease among agricultural workers.

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Organic dust inhalation in agricultural work environments has been associated with adverse respiratory responses that acutely induce organic dust toxic syndrome (ODTS), and chronically contribute to the development of asthma and asthma-like syndrome, and chronic obstructive airway disease (Linaker & Smedley, 2002; Rushton, 2007; Rylander, 2006; Seifert et al., 2003; Spurzem et al., 2002). Endotoxins are comprised of lipopolysaccharides that are components of gram-negative bacterial cell walls (Mayeux, 1997; Gutsmann et al., 2007; Rylander, 2006; Thorn, 2001). These agents are a common constituent of agricultural dusts and contribute significantly to the pathogenicity of inhaled agricultural particulates (Rushton, 2007; Rylander, 2006; Seifert et al., 2003; Thorn, 2001). Endotoxin inhalation elicits a potent innate immune response resulting in increased concentrations of cellular and soluble mediators of airway inflammation, as well as increased respiratory symptomatology and changes in quantitative measures of pulmonary function (Linaker & Smedley, 2002; Spurzem et al., 2002; Thorn, 2001). Adverse responses to inhaled endotoxin contribute to the relatively high lifetime prevalence of lung disease observed among agricultural workers (~6–15%, versus ~2–3% in nonfarming comparison populations) (Linaker & Smedley, 2002; Spurzem et al., 2002). Some agricultural workers exhibit heightened susceptibility to organic dust's respiratory effects (Castellan et al., 1987; Dosman et al., 2006; Kline et al., 1999; Schwartz, 2002), resulting in self-selection of some workers out of these occupations. Other workers may develop endotoxin tolerance (Broad et al., 2006; Hoffmann et al., 2005; Von Essen & Romberger, 2003), and these factors are thought to contribute, at least in part, to a previously described "healthy worker" effect among agricultural occupations (Bakirci et al., 2006; Post et al., 1998; Rushton, 2007). The mechanisms underlying heightened endotoxin susceptibility or the development of tolerance remain to be determined but likely involve both intrinsic (genetic traits, immune system regulation) and extrinsic factors (work-related behaviors, exposure control processes, dust composition). A better understanding of how these factors contribute to the development of adverse respiratory outcomes is needed to optimize disease prevention efforts.

Few guidelines have been established to prevent the adverse effects of agricultural dust inhalation. The American Conference of Governmental Industrial Hygienists (ACGIH) has recommended threshold limit values (TLV) for total dust (10 mg/m^3) and grain dust (4 mg/m^3, for oat, wheat, and barley) (ACGIH, 2007), although the total dust guideline does not account for the biological activity of airborne agricultural particulates. There is currently no workplace standard specifically for endotoxin exposure, although a limit of 50 endotoxin units (EU)/m^3 as an 8-h time-weighted average (TWA) was suggested (Heederik & Douwes, 1997). A total dust exposure guideline of 2.4 mg/m^3 was recommended to prevent occupational health effects among workers in swine and poultry production facilities (Donham et al., 1995; 2000, 2002; Reynolds et al., 1996), and the recommended limits for total endotoxin exposures in these work environments are 614 EU/m^3 and 900 EU/m^3, respectively (Donham et al., 1995;, 2000; Reynolds et al., 1996). These guidelines are based on 37-mm cassette total dust samplers and need to be adjusted for comparison to the Institute of Medicine (IOM) inhalable sampler (Reynolds et al., 2009). The acute respiratory effects of endotoxin in other agricultural settings, such as dairies and feedlots, have not been well characterized and no endotoxin-specific exposure guidelines have been developed for these work environments.

The biologically active component of endotoxin, lipid A, is populated by hydroxylated fatty acids of varying carbon chain lengths (Mayeux, 1997; Gutsmann et al., 2007; Larsson, 1994). Endotoxin's 3-hydroxy fatty acid (3-OHFA) moieties can be quantified in agricultural particulate matter via a sensitive and specific gas chromatography/mass spectrometry (GC/MS) method (Pomorska et al., 2007; Reynolds et al., 2005). These agents serve as reliable chemical markers of endotoxin in airborne agricultural dusts, and air monitoring

using this procedure in heterogeneous work environments may have advantages over other endotoxin assays currently in use (Laitinen et al., 2001; Reynolds et al., 2005). Agricultural dust is a complex mixture of organic and nonorganic substances, and some dust constituents may interfere with existing endotoxin assays (Dehus et al., 2006; Laitinen et al., 2001; Reynolds et al., 2005). The GC/MS measures of 3-OHFA may therefore provide more accurate information about the chemical composition and biological activity of endotoxins. However, few studies have characterized the relationship between 3-OHFA endotoxin constituents and airway inflammation among workers in different agricultural settings. In this study, personal exposure to inhalable airborne dust was quantified over one work shift among grain elevator, cattle feedlot, corn farm, and dairy workers. Endotoxin in personal airborne dust samples was measured using the recombinant factor C (rFC) assay, as well as a modified GC/MS procedure that quantified endotoxin's 3-OHFA constituents (Saito et al., 2009). Relationships between each endotoxin exposure measure, post-shift respiratory symptoms, and nasal lavage fluid inflammation markers were then examined to determine whether the 3-OHFA markers are a more sensitive indicator of inflammatory airway responses among agricultural workers than the rFC or inhalable dust measures.

SUBJECTS AND METHODS

The study population was comprised of agricultural workers in Colorado and Nebraska. In northeastern Colorado, flyers describing the study were distributed to facilities in the study region through collaborations with local agricultural organizations. Facility owners and operators were contacted to solicit managerial cooperation, and workers were recruited through group meetings at participating facilities. In Nebraska, all persons working at grain elevators and on cattle feedlots in a county in eastern Nebraska, or employed at four grain elevators owned by a major grain marketer and agricultural supplier in central Nebraska, were invited to participate in this study. The study received institutional review board approval and all participants provided informed consent in either English or Spanish. Study participation was completed over one work shift and included completion of a questionnaire to ascertain demographic and work-related information as well as post-shift respiratory symptoms; full-shift personal monitoring of airborne inhalable dust, endotoxin, and 3-OHFA concentrations; and collection of one post-shift nasal lavage fluid sample for quantification of the following inflammation measures: myeloperoxidase (MPO), interleukin 8 (IL-8), albumin, eosinophil cation protein (ECP), and differential white blood cell counts. Data were collected between the spring of 2005 and fall of 2006.

Personal breathing zone samples for inhalable particulate matter were collected using (IOM) sampling cassettes and 25mm PVC filters with a 5-μm pore size (SKC, Eighty Four, PA). Inhalable particles were defined as those collected with a median cutpoint of 100 μm in aerodynamic diameter (50% collection efficiency) (ACGIH, 2007). Personal sampling pumps (MSA, Pittsburgh, PA) were calibrated to a flow rate of 2 L/min, and calibrations were rechecked following each shift. Inhalable dust samples were analyzed by weighing the internal cassette and filter as a single unit using a Mettler MT5 balance (Mettler-Toledo, Columbus, OH). The internal cassette and filter assembly were dessicated as a unit pre- and post-weighing. Field and laboratory blanks were analyzed in a similar manner.

Each dust sample was extracted in sterile, pyrogen-free water containing 0.05% Tween-20 for 1 h at room temperature (22°C) with continuous shaking. A portion of each extract was analyzed for endotoxin using the rFC assay (Cambrex, East Rutherford, NJ) (Alwis & Milton, 2006), and another portion was lyophilized and stored at –70°C for determination of 3-OHFA endotoxin constituents via a GC/MS method modified for these environments (Saito et al., personal communication). The rFC endotoxin assay measures fluorescence generated by the enzymatic cleavage of a peptide–coumarin substrate (Alwis & Milton, 2006).

Twofold serial dilutions of endotoxin standards and sample extracts were added to a 96-well plate followed by 100 µl mixture of enzyme, buffer, and fluorogenic substrate. The plates were incubated at 37°C for 1 h and fluorescence quantified by a microtiter plate reader (Biotek Instruments FLX800TBIE, Winooski, VT) at excitation/emission frequencies of 380/440 nm. Log fluorescence was proportional to log endotoxin concentration and was linear from 0.01 to 10 EU/ml. Four assay reagent blanks were used to control for the pyrogen-free status of the reagent water, centrifuge tubes, pipette tips, and microplates. Quality assurance assays were performed at multiple dilutions with and without spikes to assess matrix interference or enhancement. Initially, at least three dilutions were used to identify the expected range for each sample. Samples were then assayed at two dilutions, in triplicate. Results from different dilutions were examined for inconsistencies and none were observed at the final dilutions used in the assay. The minimum detection limit was ~0.01 EU/ml.

To quantify 3-OHFA, a GC/MS-MS method developed for house dust (Saraf & Larsson, 1996; Saraf et al., 1997, 1999; Sebastian & Larsson, 2003) was modified using a gas chromatography/electron impact mass spectrometry (GC/EI-MS) method to accommodate the small amounts (<1 mg) of agricultural dust collected via personal air monitoring for this study (Saito et al., 2009). Samples and standards derivatized with *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) were analyzed using an HP 5890 Series II Plus gas chromatograph equipped with an HP-5MS column (0.25 mm × 30 m, 0.25 µm film thickness, Hewlett-Packard, Palo Alto, CA), and an HP 5972 Mass Selective Detector. Selected ion monitoring was used for individual 3-OHFA, and results were quantified in picomoles. Calibration was accomplished via laboratory-fortified matrix blanks at anticipated 3-OHFA concentrations in dust samples (Saito et al., personal communication). The limit of detection (LOD) and the limit of quantitation (LOQ) were determined by signal-to-noise (S/N) ratio based on the chromatograms of controls and 0.5- and 1-ng spikes (S/N > 3 for LOD and S/N > 10 for LOQ) (Mac Dougull, 1980).

Structured, self-administered questionnaires were completed in Spanish or English before and after each participant's work shift in order to ascertain information on individual demographic and lifestyle factors (age, socioeconomic status, race/ethnicity, tobacco and alcohol consumption, medication use, farm as residence), job and workplace characteristics (job title and work duration, frequency and duration of work on farms and in grain elevators), potential workplace exposure to hazardous or toxic substances (visible dust, metal fumes, gases/vapors, pesticide use, respirator use), and respiratory health (allergies, asthma, chronic bronchitis, emphysema, lung cancer, cold/flu, sinus problems, pneumonia, anosmia). Questions used to characterize ODTS were also based on a previously developed instrument (Rylander et al., 1990). Chronic respiratory symptoms (cough, phlegm, wheezing, shortness of breath, nasal irritation, fever/chills) were ascertained using questions from the American Thoracic Society's standardized instrument (Ferris, 1978). The severity of each participant's post-work-shift respiratory symptoms (eye, nose, or throat irritation; shortness of breath; phlegm; headache; wheezing; cough) was assessed using a five-level nominal scale (none, mild, moderate, severe, very severe). The Spanish translation of this questionnaire was pilot tested among Spanish-speaking agricultural workers prior to use in the study.

A nasal lavage fluid sample was collected at the end of each participant's work shift using a previously described protocol (Naclerio et al., 1983; Saito et al., 2009). Pre-shift nasal lavages were not performed because cross-shift comparisons of lavage constituents are susceptible to artifact (Hauser et al., 1994). To collect post-shift samples, a 5-ml volume of normal (0.9%) saline was instilled into each nostril of a seated subject with the person's neck extended back. Subjects were instructed not to breathe or swallow. After 10 s, subjects flexed their neck forward approximately 30° from horizontal to expel the saline into a sterile container.

Specimens were kept on ice for no longer than 30 min, transferred to 50-cc centrifuge tubes, and centrifuged at 1500 rpm for 10 min at room temperature. The supernatant fluid was then divided into 1-ml aliquots and kept on ice until frozen at –70ºC. The cell pellet was resuspended in 30 ml Cytolyte mucolytic preservative solution (Cytyc Corp., Marlborough, MA). These samples were shipped via express mail to the National Institute for Occupational Safety and Health (NIOSH) Bio-Organic Chemistry Laboratory, where they were centrifuged at 600 × g for 10 min, and the cell pellet was resuspended in 20 ml PerservCyt (Cytyc Corp., Marlborough, MA, ISO registration: FM 503552) and stored at 4°C. Cytology slides were prepared using the ThinPrep instrument (Cytyc Corp., Marlborough, MA), and the amount of sample not transferred to the cytology slide was measured and recorded. Each slide was stained with the Diff-Quick stain kit (Lmeb, Inc., San Marcos, CA). The nasal lavage cell numbers and differentials were evaluated in 20 visual microscopic fields under oil (500×). The cell numbers were adjusted for total cell spot area and percent of nasal lavage sample transferred onto the microscope slide to provide cell number per nasal lavage.

Frozen nasal lavage fluid supernatants were shipped on dry ice to the NIOSH Bio-Organic Chemistry Laboratory. All samples arrived frozen, were transferred into a –80ºC freezer until analysis, and did not undergo repeated freeze–thaws. Lavage supernatants were analyzed using commercially available immunoassay kits in accordance with each manufacturer's standardized assay protocol. The following inflammation marker proteins were quantified: myeloperoxidase (MPO; Assay Designs, Inc., Ann Arbor, MI); ECP (Phadia, Portage, MI, formerly Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden); albumin (Bethyl Laboratories, Montgomery, TX); and interleukin-8 (IL-8; R&D Systems, Minneapolis, MN) (Gaughan et al., 2008; Woodin et al., 1998). These assays were previously evaluated for interferences by nasal lavage constituents and none were observed. All samples for a single analyte were assayed as a batch using the same kit to avoid lot to lot variability. ECP and IL-8 were assayed undiluted. For MPO and albumin, lavage fluid was initially diluted with assay buffer 10-fold and 100-fold, respectively. When required, samples were diluted with their respective assay diluent and re-analyzed to achieve analyte concentrations within the quantitative range of the assay. Samples with inflammation marker concentrations that were not quantifiable were assigned a value of one-half the limit of quantification. The limits of quantification were 500 pg/ml, 630 pg/ml, 1.6 pg/ml, and 6.25 ng/ml for ECP, MPO, IL-8, and albumin, respectively.

Statistical analyses were performed using the SAS computer program (version 9.1, SAS Institute, Inc., Cary, NC). Endotoxin and 3-OHFA concentrations were normalized either to the total amount of dust sampled (EU/mg or pmol/mg, respectively) or to the volume of air sampled (EU/m^3 or pmol/m^3, respectively). Analyses were performed separately for the sum of all 3-OHFA constituents measured and for the sum of the even- or odd-numbered 3-OHFA. Results for the C16:0 3-OHFA were excluded from the analysis due to assay interference and because 61 of 125 samples (49%) had undetectable C16:0 levels. Exposure and inflammation marker variables were analyzed using log-transformed data; back-transformations were performed for tabular presentation of geometric mean values. To evaluate respiratory symptoms, individuals were grouped according to the presence (any severity score ≥1) or absence of each post-shift symptom, and the generalized linear models (GLM) procedure in SAS was used to compare mean dust, endotoxin or inflammation marker levels within each group. The proportions of individuals with post-shift respiratory symptoms among quartiles of work-shift dust or endotoxin exposure were also computed, and proportions in the first and fourth quartile were compared using the Mantel–Haenzel chi-squared test statistic. The proportion of symptoms among those with exposures above or below potential thresholds previously suggested by other investigators, namely, 300 EU/m^3 (25 ng/m^3) (Laitinen et al., 2001) and 2400 EU/m^3 (200 ng/m^3) (Rylander,

1997), were evaluated similarly. Relationships among inflammation markers or among exposure measures were evaluated by calculating Spearman rank correlation coefficients (r). The relationship between various participant or workplace characteristics and mean inflammation marker concentrations was evaluated by computing mean concentrations among workers grouped at each level of a given characteristic with the GLM procedure; differences between means were compared using the least significant difference (LSD) method.

To test the hypothesis that workplace inhalable dust, endotoxin, or 3-OHFA exposure was associated with increased inflammation marker concentrations in nasal lavage fluids, individuals were grouped into dust or endotoxin exposure quartiles, and the GLM procedure was used to compute adjusted (least squares or LS) means of inflammation marker concentrations within each quartile. A two-stage variable selection procedure was used to identify questionnaire items associated with each exposure parameter for inclusion in the statistical models as potential confounding factors. First, items were univariately compared with each exposure measure using the GLM procedure. Next, all variables associated with a given exposure variable ($p \leq .1$) were combined in a single model, and backward elimination was used to identify a final list of covariates ($p \leq .05$). The GLM procedure was then used to combine these variables along with an exposure measure in a statistical model, and adjusted means of inflammation markers in the upper and lower exposure quartiles were compared using the LSD method. To test for linear trend, each exposure parameter was evaluated as a continuous variable in separate GLM analyses, with adjustment for the selected covariates. Effect modification was assessed by stratifying workers within each exposure quartile into groups above and below the median of another exposure variable obtained via the questionnaire. These analyses focused on items potentially linked with chronic exposure to organic dust (work duration in current job; frequency or duration of work on farms or in grain elevators/corn storage areas). Adjusted means were calculated within each stratum of exposure and means in the lowest and highest exposure quartiles were compared statistically, as described above. Means from the upper exposure quartiles were also compared across the strata of each chronic exposure parameter using the same methods.

RESULTS

There were 174 participants from 26 work sites originally recruited into the study; approximately 350 workers were contacted and the participation rate was approximately 50%. Relationships between personal endotoxin exposures and post-shift nasal lavage inflammation markers or symptoms were evaluated among 125 men with complete data available for analysis (18 subjects without a nasal lavage sample, 27 subjects without personal air monitoring data, 4 females excluded due to small group size). Characteristics of the study population are presented in Table 1. The average (± standard deviation) age was 34 ± 11 yr, and most participants were Caucasian (70%) and non-Hispanic (58%). The study population included 46 grain handlers (37%), 55 cattle feedlot workers (43%), 15 dairy workers (12%), and 9 corn farmers (8%) (Table 1). The average duration of employment (in current job) was 7 ± 8.5 yr.

Inhalable dust concentrations were moderately correlated with endotoxin or 3-OHFA measures in air (r = approximately .4 to .6), were uncorrelated with endotoxin concentrations in dust (EU/mg, r = .07), and were weakly negatively correlated with 3-OHFA concentrations in dust (r = –.2 to –.5). There were moderate positive correlations between endotoxin (rFC) and 3-OHFA measures (r = .5 to .7), and correlation coefficients for relationships between even and odd 3-OHFA measures were approximately .7.

An exposure guideline of 2.4 mg/m^3 for total dust corresponds to an occupational exposure guideline of approximately 4.8 mg/m^3 for studies using IOM samplers in these environments (Reynolds et al., 2009). The geometric mean time-weighted average (TWA) for

TABLE 1. Population Characteristics (n = 125)

Characteristic	n	Percentage
Age distribution		
18–24 years	37	29%
25–40 years	57	46%
41–72 years	31	25%
Race		
Caucasian	87	70%
Native or Mexican American	32	26%
Ethnicity		
Hispanic or Latino	43	34%
Not Hispanic or Latino	73	58%
English education		
None	35	28%
Primary/secondary only	1	1%
High school	45	36%
College	44	35%
Spanish education		
None	90	72%
Primary/secondary	33	26%
High school or college	2	2%
Tobacco use		
Never	64	51%
Former	13	10%
Current:	47	38%
Daily cigarette/cigar smoker	31	25%
Daily chew/snuff use only	16	13%
Job status		
Owner/family member	22	18%
Local hire	85	68%
Seasonal or temporary worker	17	14%
Type of facility		
Grain elevator	46	37%
Cattle feedlot	55	43%
Dairy	15	12%
Corn farm	9	8%
Respirator use		
Respirator with filters	5	4%
Dust mask/surgeon's mask	14	11%
Bandana/scarf	0	0%
None of the above	103	84%
Job duration		
<1 yr	14	11%
1.0–3.9 yr	47	38%
4.0–7.9 yr	28	22%
≥8 yr	34	27%
Subject resides at the work site		
Yes	24	19%
No	101	81%
Subject lives on a farm		
Yes	45	36%
No	78	62%
Livestock kept at work site		
Yes	75	60%
No	49	39%
Post-shift symptoms		
Eye irritation	41	33%
Nose irritation	42	34%
Throat irritation	23	18%
Mucous or phlegm	42	34%
Shortness of breath	14	11%
Headache	12	10%
Chest wheezing or whistling	8	6%
Cough	33	26%

Note. Percentages totaling <100% are due to refusals or missing data.

inhalable dust exposures among all participants was 3.4 mg/m^3 (geometric standard deviation [GSD] = 3), and the recommended inhalable dust guideline (4.8 mg/m^3) was exceeded among approximately 33% of participants. The geometric mean TWA of endotoxin exposure among all participants was 888 (6.5) EU/m^3, and the suggested endotoxin exposure limit (50 EU/m^3) was exceeded by 93% of the study participants. Swine and poultry production facilities were not included in this study, but for comparison the recommended limits for total endotoxin exposure in these work environments (Donham et al., 1995, 2000, 2002; Reynolds et al., 1996) were exceeded by 47% and 37% of the study subjects, respectively. Table 2 presents geometric mean TWA concentrations of personal dust, endotoxin, and total, even-numbered, and odd-numbered 3-OHFA exposure measures, along with mean even:odd 3-OHFA ratios by work facility. Grain elevators had the highest levels of inhalable dust and the highest even:odd 3-OHFA ratios. Note that the rank ordering among the facilities differs with respect to the dust, endotoxin, and 3-OHFA measures.

Among the cellular subsets that were quantified for differential cell counts, only polymorphonuclear neutrophils (PMN) were detected in sufficient quantities to allow statistical comparisons (n = 9 samples below detection). The nondetection rates for lymphocytes, monocytes, eosinophils and basophils were 66%, 89%, 89%, and 100%, respectively. Correlations among the nasal lavage inflammation markers are presented in Table 3. ECP was moderately correlated with each of the other inflammation markers, and IL-8 was modestly

TABLE 2. Inhalable Dust, Endotoxin (rFC Assay), or 3-OHFA Personal Exposures[a] by Facility

Facility	Dust (mg/m^3)	Endotoxin (EU/m^3)	Total 3-OHFA ($pmol/m^3$)	Even 3-OHFA ($pmol/m^3$)	Odd 3-OHFA ($pmol/m^3$)	Even/odd 3-OHFA ratio
Grain elevator (n = 46)	5.6 (3.8)[b,c]	813 (7.9)	1787 (5.5)	1434 (5.8)	195 (3.5)	7.4 (3.9)[b,c]
Cattle feedlot (n = 55)	2.4 (2.1)	943 (7.1)	2370 (4.1)	1756 (4.2)	518 (3.8)[d]	3.4 (2.3)
Dairy (n = 15)	2.4 (1.9)		1357 (2.6)	1050 (2.7)	247 (3.1)	4.3 (2.1)
Corn farm (n = 9)	2.9 (3.6)	625 (4.5)	2265 (2.1)	1598 (2.2)	447 (3.4)	3.6 (3.5)

[a]Geometric mean (geometric standard deviation).
[b]Significant at $p \leq .05$ vs. feedlots.
[c]Significant at $p \leq .05$ vs. dairies.
[d]Significant at $p \leq .05$ vs. grain elevators.

TABLE 3. Correlations Among Inflammation Markers

	PMN (cells/ml)	MPO (ng/myl)	IL-8 (pg/ml)	Albumin (ng/ml)	ECP (ng/ml)
PMN (cells/ml)	1.00	0.18 (.05)	0.27 (<.01)	0.30 (<.01)	0.52 (<.01)
MPO (ng/ml)	—	1.00	0.08 (.38)	0.09 (.31)	0.44 (<.01)
IL-8 (pg/ml)	—	—	1.00	0.34 (<.01)	0.32 (<.01)
Albumin (ng/ml)	—	—	—	1.00	0.36 (<.01)
ECP (ng/ml)	—	—	—	—	1.00

Note. Correlations given as Spearman rank correlation coefficients (p values in parentheses). PMN, polymorphonuclear neutrophils. MPO, myeloperoxidase. IL, interleukin. ECP, eosinophilic cation protein.

correlated with PMN and albumin (Table 3). MPO was not correlated with IL-8 or albumin, and only weakly correlated with PMN (Table 3).

Table 4 presents unadjusted mean inflammation marker concentrations for various characteristics of the study population. Mean MPO levels were greater among Caucasians and non-Hispanics and rose with increasing age, job duration, or duration of farm work. Mean MPO was also markedly greater among participants reporting home pesticide use within the past month (117 vs. 35 pg/ml, Table 4). Nasal lavage fluid IL-8 concentrations were elevated among workers in cattle feedlots, dairies, and corn farms compared to those working in grain elevators; among workers with a longer duration of farm work; and among those located at work sites where livestock was kept (242 vs. 153 pg/ml, Table 4). Mean IL-8 levels were more than doubled among those who applied Vaseline or water to their noses to reduce dust exposures or ameliorate nasal irritation compared to those who didn't (419 vs. 199 pg/ml, Table 4). An increased frequency or duration of work in grain elevators or corn storage areas was associated with a reduction in IL-8 concentrations (Table 4). A similar pattern of reductions in mean albumin levels was observed among workers with an increased frequency but not duration of grain elevator or corn storage work (Table 4). Mean albumin levels were greater among Caucasians and non-Hispanics, and among those with an increased frequency or duration of farm work (Table 4). ECP concentrations tended to rise with longer job duration or with a greater frequency or duration of farm work (Table 4).

There were no statistically significant changes in post-work-shift symptom prevalence (eye, nose, or throat irritation; shortness of breath; phlegm; headache; wheezing; cough) among workers grouped according to quartiles of dust, endotoxin, or 3-OHFA exposure (data not shown). Similarly, there were no statistically significant differences in adjusted geometric mean TWA dust, endotoxin, or 3-OHFA exposures among participants with or without a given symptom (data not shown). The proportion of symptoms among those with exposures above and below potential thresholds of 300 EU/m^3 or 2,400 EU/m^3 also did not differ (data not shown). In addition, there were

TABLE 4. Mean Inflammation Marker Levels by Population Characteristic (n = 125)

Characteristic	PMN (cells/ml)	MPO (ng/ml)	IL-8 (pg/ml)	Albumin (ng/ml)	ECP (ng/ml)
Age group†	(.62)	(.05)	(.19)	(.39)	(.25)
18–24 yr (n = 37)	446	30	245	6634	0.99
25–40 yr (n = 57)	545	40	181	4447	1.07
41–72 yr (n = 31)	665	55	200	6003	1.16
Race					
Caucasian (n = 87)	536	50*	196	6843*	1.21
Native or Mexican American (n = 32)	503	28	204	3294	0.85
Ethnicity					
Hispanic or Latino (n = 43)	491	27	204	3323	0.86
Not Hispanic or Latino (n = 73)	542	53*	189	6853*	1.22
Tobacco use					
Never (n = 64)	620	47	199	6674	1.09
Former (n = 13)	1404	46	211	2736*	1.82
Current (n = 47):	347[a]	33	202	4978	0.91[a]
Daily cigarette/cigar smoker (n = 31)	219[a,]*	40	190	4505	0.89
Daily chew/snuff use only (n = 16)	846[b,]*	23	229	6041	0.95
Type of facility					
Grain elevator (n = 46)	438	33	154	5110	0.93
Feedlot (n = 55)	528	50	221[c]	5715	1.11
Dairy (n = 15)	744	41	245[c]	5385	1.07
Farm (n = 9)	992	32	390[c]	6048	1.69
Hours worked week of participation†	(.21)	(.51)	(.64)	(.51)	(.52)
<10 h (n = 31)	363	45	180	4182	0.79
11–24 h (n = 32)	657	49	221	7268	1.36*
25–40 h (n = 33)	801	29	199	4667	1.00
>40 h (n = 28)	476	42	214	6391	1.22
Job duration†	(.65)	(.86)	(.38)	(.35)	(.28)
<1 yr	256	23	208	6503	0.85
1.0–3.9 yr	370*	38	226	5714	0.88
4.0–7.9 yr	994*	44	182	5526	1.18
≥8 yr	713	54*	189	4812	1.43[d]
Frequency of farm work†	(.43)	(.18)	(.02)	(.11)	(.17)
None (n = 34)	435	38	149	3580	0.90
<1–10 months per year (n = 23)	454	25	220	7218*	0.81
12 months per year (n = 62)	559	49	225*	5978*	1.20
Duration of farm work†	(.93)	(.03)	(.13)	(.07)	(.19)
None (n = 25)	571	28	143	3305	0.75
< 1 – 4.9 yr (n = 21)	269	31	218	5429	0.74
≥ 5 yr (n = 72)	675	52*	229*	6678*	1.36*
Frequency of grain elevator or corn storage work†	(.49)	(.31)	(.09)	(.04)	(.56)
None (n = 42)	566	37	249	6755	1.03
<1–10 months per year (n = 39)	707	30	178	6023	1.17
12 months per year (n = 38)	377	55	173*	3824*	0.96
Duration of grain elevator or corn storage work†	(.91)	(.17)	(.36)	(.75)	(.41)
None (n = 46)	683	36	266	5136	1.15
<1–4.9 yr (n = 37)	335	32	162*	6088	0.84
≥5 yr (n = 36)	688	53	172*	5107	1.24
Livestock kept at work site					
No (n = 49)	476	35	153	5055	0.99
Yes (n = 75)	574	45	242*	5710	1.10
Home pesticide use within 1 month					
No (n = 106)	554	35	211	5499	1.03
Yes (n = 17)	427	117*	151	5301	1.31
Nasal treatment with water or petroleum jelly					
No (n = 118)	542	43	199	5426	1.04
Yes (n = 5)	734	40	419*	9210	2.07

Note. Significance indicated by *$p \leq .05$ vs. referent group (first row appearing for each characteristic unless noted); †, p value for characteristic as a continuous variable in parentheses; a, $p \leq .05$ vs. former tobacco users; b, $p \leq .05$ vs. current cigarette/cigar smoker; c, $p \leq .05$ vs. workers in grain elevators; d, $p \leq .05$ vs. 1.0–3.9 yr group. Frequencies less than n = 125 are due to refusals or missing data. PMN, polymorphonuclear neutrophils. MPO, myeloperoxidase. IL, interleukin. ECP, eosinophilic cation protein.

no differences in the frequency of respirator use among the different exposure groups studied (data not shown). However, mean nasal lavage PMN counts (1672 vs. 476 cells/ml), ECP (2.55 vs. 0.97 ng/ml), or albumin concentrations (12,922 vs. 4978 ng/ml) were elevated among those reporting post-shift headaches compared to those without headaches. Mean albumin levels were also higher among those with excessive phlegm following their work shift (7352 vs. 4711 pg/ml). Measurable increases in mean lavage fluid ECP concentrations were noted among participants with post-shift cough (1.45 vs. 0.96 pg/ml).

Table 5 presents adjusted means of nasal lavage fluid inflammation markers among quartiles of personal TWA exposure to inhalable dust, endotoxin, or its 3-OHFA constituents. There were unexpected decreases in PMN, MPO, and ECP levels (as continuous variables) in response to increasing TWA inhalable dust exposures. When normalized to either the total weight of dust sampled (EU/mg) or the volume of air sampled (EU/m^3), endotoxin exposures were associated with statistically significant increases in IL-8 concentrations. However, there were no other statistically significant changes in inflammatory markers in response to the endotoxin measures (Table 5). When endotoxin exposures were characterized using the sum of the 3-OHFA (pmol/mg), there were statistically significant elevations in PMN, MPO, and ECP with increasing 3-OHFA exposure (Table 5). Some nasal lavage inflammation markers were also elevated in response to total 3-OHFA normalized to the air volume sampled (pmol/m^3), although only changes in mean MPO attained statistical significance (Table 5). The most robust increases in lavage fluid inflammation markers were observed in response to even-numbered carbon chain length 3-OHFA. Workers in the upper quartile of even-numbered 3-OHFA (pmol/mg) exposures had adjusted mean PMN and MPO levels that were approximately threefold greater and mean albumin and ECP concentrations that were more than double those observed among workers in the lowest exposure quartile (Table 5). Results obtained using the even-numbered 3-OHFA concentrations as continuous variables were consistent with results obtained using exposure quartiles (Table 5). When odd-numbered 3-OHFA were evaluated (either pmol/m^3 or pmol/mg), there were no statistically significant changes in post-shift inflammation marker levels (Table 5).

When study subjects within the total 3-OHFA quartiles (pmol/m^3) were further stratified by other exposure variables from the questionnaire (current job duration; frequency or duration of work on farms or in grain elevators/corn storage areas), the results tended to vary both by the type of inflammation marker being evaluated and by the stratification variable (Table 6). An increased frequency or duration of work in grain elevators or corn storage areas tended to enhance the effects of 3-OHFA exposure on PMN and MPO, whereas IL-8 and ECP concentrations were not altered significantly (Table 6). Albumin concentrations were enhanced by a shorter duration of work in grain elevators or corn storage areas (Table 6). Workers with fewer years of work on farms tended to have elevated PMN, MPO, albumin, ECP, and to a lesser extent IL-8 levels in response to 3-OHFA exposures (Table 6). Stratification by median job duration (4 yr) exerted a negligible impact on the relationship between 3-OHFA exposures and the inflammation markers studied (data not shown). Adjusted mean inflammation markers were also evaluated by facility, as well as relationships between personal exposures and inflammation markers with stratification by facility. Albumin tended to be elevated among workers in grain elevators, but no other noteworthy differences were observed (data not shown).

DISCUSSION

Exposure of agricultural workers to organic dust has been associated with increased airway inflammation, decrements in respiratory function, and debilitating respiratory diseases including ODTS, rhinitis, asthma and asthma-like syndrome, and chronic obstructive pulmonary disease (Linaker & Smedley, 2002; Rushton, 2007; Rylander, 2006; Seifert et al., 2003;

TABLE 5. Inflammation Marker Levels by Inhalable Dust, Endotoxin (rFC Assay), or 3-OHFA Exposure

Inflammation marker	Exposure quartiles				1 vs. 4 *p* value	1 vs. 4 Difference	Continuous variable *p* value
	1	2	3	4			
	Dust[a] (mg/m^3)						
	1 (2)	2 (1)	4 (1)	14 (2)			
PMN (cells/ml)	797	632	480	366	.18	−54%	.05
MPO (ng/ml)	57	41	43	21	.01	−63%	.03
IL-8 (pg/ml)	144	270	190	214	.11	49%	.13
Albumin (ng/ml)	4022	4418	8014	6334	.22	57%	.77
ECP (ng/ml)	1.16	1.14	1.27	0.74	.18	−36%	.06
	Endotoxin[b] (EU/mg)						
	46 (2)	161 (1)	422 (1)	1679 (2)			
PMN (cells/ml)	447	593	672	447	.99	0%	.72
MPO (ng/ml)	39	25	65	37	.91	−5%	.83
IL-8 (pg/ml)	145	177	258	228	.05	63%	.01
Albumin (ng/ml)	3137	3436	5491	5839	.06	86%	.14
ECP (ng/ml)	0.81	1.17	1.29	0.90	.60	11%	.49
	Endotoxin[c] (EU/m^3)						
	79 (2)	473 (2)	1870 (1)	9354 (2)			
PMN (cells/ml)	657	432	638	614	.90	−6%	.74
MPO (ng/ml)	57	59	71	63	.81	11%	.77
IL-8 (pg/ml)	175	164	245	241	.18	38%	.04
Albumin (ng/ml)	4008	3950	5429	5548	.34	38%	.59
ECP (ng/ml)	1.35	1.46	0.92	1.36	.99	0%	.66
	Sum of all 3-OH fatty acids[d] (pmol/mg)						
	137 (2)	374 (1)	904 (1)	3172 (2)			
PMN (cells/ml)	359	317	808	849	.11	136%	.01
MPO (ng/ml)	21	32	65	53	.01	152%	.02
IL-8 (pg/ml)	216	145	191	255	.48	18%	.35
Albumin (ng/ml)	4292	5480	5150	6721	.20	57%	.31
ECP (ng/ml)	0.74	1.03	1.22	1.45	.03	96%	.05
	Sum of all 3-OH fatty acids[e] (pmol/m^3)						
	335 (2)	1251 (1)	3236 (1)	13,325 (2)			
PMN (cells/ml)	535	284	336	824	.39	54%	.34
MPO (ng/ml)	21	28	38	50	.03	138%	.06
IL-8 (pg/ml)	176	193	192	259	.11	47%	.13
Albumin (ng/ml)	3445	5509	4724	6210	.08	80%	.10
ECP (ng/ml)	1.09	0.67	0.77	1.42	.40	30%	.31
	Sum of even 3-OH fatty acids[f] (pmol/mg)						
	103 (2)	297 (1)	737 (1)	2705 (2)			
PMN (cells/ml)	299	602	461	992	.03	232%	.02
MPO (ng/ml)	20	36	56	59	<.01	195%	.01
IL-8 (pg/ml)	168	187	218	226	.21	34%	.44
Albumin (ng/ml)	3350	6764	5412	6829	.04	104%	.19
ECP (ng/ml)	0.72	1.25	0.98	1.57	.01	118%	.03

(*Continued*)

TABLE 5. (*Continued*)

Inflammation marker	Exposure quartiles				1 vs. 4 *p* value	1 vs. 4 Difference	Continuous variable *p* value
	1	2	3	4			
	Sum of odd 3-OH fatty acids[g] (pmol/mg)						
	17 (2)	66 (1)	156 (1)	553 (2)			
PMN (cells/ml)	404	383	740	877	.24	117%	.09
MPO (ng/ml)	33	23	34	54	.26	64%	.48
IL-8 (pg/ml)	210	248	125	211	.99	1%	.72
Albumin (ng/ml)	8781	5952	4287	4822	.15	–45%	.20
ECP (ng/ml)	1.18	1.61	1.43	1.13	.90	–4%	.97
	Sum of even 3-OH fatty acids[h] (pmol/m^3)						
	246 (2)	1021 (1)	2600 (1)	12,681 (3)			
PMN (cells/ml)	805	330	347	922	.79	15%	.20
MPO (ng/ml)	34	32	38	67	.07	97%	.16
IL-8 (pg/ml)	183	153	250	242	.21	32%	.48
Albumin (ng/ml)	4210	6501	4801	6342	.23	51%	.36
ECP (ng/ml)	1.51	0.63	1.13	1.44	.87	–5%	.11
	Sum of odd 3-OH fatty acids[i] (pmol/m^3)						
	66 (2)	163 (1)	488 (1)	1772 (2)			
PMN (cells/ml)	1103	283	945	524	.19	–52%	.59
MPO (ng/ml)	36	38	45	34	.84	–6%	.85
IL-8 (pg/ml)	189	218	196	210	.66	11%	.21
Albumin (ng/ml)	8978	4818	6177	5883	.25	–34%	.44
ECP (ng/ml)	1.57	1.76	1.65	1.13	.32	–28%	.93

Note. Exposure quartiles are given as geometric mean (geometric standard deviation). PMN, polymorphonuclear neutrophils. MPO, myeloperoxidase. IL, interleukin. ECP, eosinophilic cation protein. Least squares means are presented with adjustment for: a, days per year working in a grain elevator or corn storage bin, hours worked week of study; b, Spanish education, hours worked week of study, months of farm work per year; c, Spanish education, job status, home pesticide use, hours worked week of study; d, months working in grain elevator or corn storage bin; e -,hours worked week of study, English education, daily tobacco use; f, months working in grain elevator or corn storage bin; g, livestock on farm, feeding livestock, job status, months working in grain elevator or corn storage bin; h, no covariates identified; i, job status, livestock on farm, feeding of livestock, daily ibuprofen use.

Spurzem et al., 2002). The mechanism underlying these disorders involves immune system activation (Thorn, 2001), although the relative contributions of specific work environments, work-related behaviors, individual susceptibilities, and different dust constituents remain to be fully elucidated. Endotoxin and 3-OHFA are components of a complex environment that may be responsible for inflammatory responses induced by agricultural dust exposures. Heterogeneity in lipid A composition has been associated with differences in bacterial species and with variations in immunological potency (Akira et al., 2006; Helander et al., 1982; Miller et al., 2005). To our knowledge, this was the first study to evaluate the relationship between endotoxin's 3-OHFA constituents in agricultural dust and human nasal airway inflammation. Results indicated that the 3-OHFA were associated with a two- to three-fold increase in mean nasal lavage fluid PMN, MPO, albumin, and ECP levels. Responses associated with the 3-OHFA were greater than those associated with another endotoxin measure (rFC) or with inhalable dust exposures. These findings are consistent with other observations suggesting that the 3-OHFA may provide a more biologically relevant exposure measure than previously described endotoxin assays (Dehus et al., 2006). This study did not evaluate respiratory disease outcomes, but the results may have implications for the

TABLE 6. Inflammation Marker Levels by 3-OHFA (pmol/m^3) and Chronic Exposure Parameters

Inflammation Marker	Exposure quartiles					
	1	2	3	4		
	Sum of all 3-OH fatty acids[a] (pmol/m^3)					
	335 (2)	1251 (1)	3236 (1)	13,325 (2)	1 vs. 4 *p* value	1 vs. 4 Difference
<3 mo per year working at grain elevator or corn storage area						
PMN (cells/ml)	525	379	327	422	.78	–20%
MPO (ng/ml)	15	28	24	42	.09	180%
IL-8 (pg/ml)	169	225	258	254	.29	50%
Albumin (ng/ml)	5270	6028	5944	9204	.29	75%
ECP (ng/ml)	1.50	0.67	0.95	1.20	.64	–20%
≥3 mo per year working at grain elevator or corn storage area						
PMN (cells/ml)	519	158	365	1225	.18	136%
MPO (ng/ml)	24	23	38	56	.06	133%
IL-8 (pg/ml)	169	129	129	262	.14	55%
Albumin (ng/ml)	2670	5216	3702	4871	.15	82%
ECP (ng/ml)	0.91	0.53	0.58	1.59	.13	75%
<2 yr working at grain elevator or corn storage area						
PMN (cells/ml)	463	317	590	566	.80	22%
MPO (ng/ml)	29	27	31	34	.78	17%
IL-8 (pg/ml)	224	211	242	257	.72	15%
Albumin (ng/ml)	2534	5821	7467	8374	.02	230%
ECP (ng/ml)	1.52	0.79	0.86	1.23	.67	–19%
≥2 yr working at grain elevator or corn storage area						
PMN (cells/ml)	529	221	181	1,162	.24	120%
MPO (ng/ml)	17	26	29	75	<.01	341%
IL-8 (pg/ml)	147	150	136	261	.07	78%
Albumin (ng/ml)	3609	4332	2689	4549	.59	26%
ECP (ng/ml)	0.89	0.48	0.62	1.64	.12	84%
<12 mo per year working on a farm						
PMN (cells/ml)	463	326	282	1471	.09	218%
MPO (ng/ml)	17	21	32	63	.02	271%
IL-8 (pg/ml)	155	155	188	267	.11	72%
Albumin (ng/ml)	2266	7978	5051	5544	.05	145%
ECP (ng/ml)	0.78	0.66	0.57	1.80	.04	131%
≥12 mo per year working on a farm						
PMN (cells/ml)	598	250	425	454	.73	–24%
MPO (ng/ml)	32	39	49	38	.80	19%
IL-8 (pg/ml)	238	229	206	245	.94	3%
Albumin (ng/ml)	6202	4490	4513	6717	.88	8%
ECP (ng/ml)	2.12	0.65	1.10	1.12	.15	–47%
<10 yr working on a farm						
PMN (cells/ml)	536	393	305	2695†	.03	403%
MPO (ng/ml)	18	21	27	39	.14	117%
IL-8 (pg/ml)	190	171	178	286	.26	51%
Albumin (ng/ml)	2562	6787	5982	7300	.04	185%
ECP (ng/ml)	0.78	0.72	0.72	1.91	.06	145%
≥10 yr working on a farm						
PMN (cells/ml)	653	206	437	351	.39	–46%
MPO (ng/ml)	36	33	61	44	.73	22%
IL-8 (pg/ml)	185	241	221	233	.52	26%
Albumin (ng/ml)	5858	4302	3607	5480	.89	–6%
ECP (ng/ml)	1.85	0.59	0.94	1.14	.28	–38%

Note. Exposure quartiles are given as geometric mean (geometric standard deviation). Significant difference indicated by †, $p \leq .05$ for comparison of upper quartiles. Least squares means are presented with adjustment for: hours worked week of study, English education, daily tobacco use. PMN, polymorphonuclear neutrophils. MPO, myeloperoxidase. IL, interleukin. ECP, eosinophilic cation protein.

development of respiratory disease prevention strategies among agricultural workers. Even-numbered carbon chain length 3-OHFA were associated with a robust response, whereas odd-numbered 3-OHFA were not associated with notable changes in nasal lavage fluid inflammation markers. This suggests that the odd-numbered 3-OHFA may be less potent, possibly due to differences in their binding properties and their affinity to 3-OHFA (Toll-like) receptors. More research is required to evaluate this possibility (Broad et al., 2006; Gutsmann et al., 2007). Studies in experimental systems indicate that 3-OHFA molecules 12 to 14 carbons in length elicit the most potent immunological effects (Dehus et al., 2006).

In general, our findings are consistent with previous studies that documented enhanced PMN recruitment (Sigsgaard et al., 2000) and elevated IL-8 (Sigsgaard et al., 2000), MPO (Heldal et al., 2003), and albumin (Sigsgaard et al., 2000) in nasal lavage fluids from subjects exposed to endotoxin in agricultural or other work environments, or in controlled laboratory settings. Neutrophil infiltration is an established response to nasal irritation by agents such as endotoxin and is mediated via the chemoattractant IL-8 (Kobayashi, 2008). The effects of PMN are carried out in part through the release of the pro-oxidant enzyme MPO (Kobayashi, 2008; Thorn, 2001). ECP is a mediator of allergic inflammation that is elevated in human sputum following endotoxin exposure (Michel et al., 1997; Thorn & Rylander, 1998). It is not possible to fully assess the contribution of allergic rhinitis to the endotoxin responses observed in the present data. ECP, while found in high concentrations in eosinophil granules, has also been characterized in neutrophils (Monteseirin et al., 2007). The low eosinophil cell content in this study's nasal lavage samples suggests that acute allergic rhinitis was not a major contributor to the observed nasal inflammation.

Because the study population represented a convenience sample, bias could have been introduced if nonparticipants differed from participants in a manner that influenced the exposure-response relationships that were evaluated. Potential reasons for nonparticipation likely varied from simple inconvenience to potential exposure-related issues. Exposures among our study participants were clearly elevated. The inhalable dust guideline was exceeded among approximately 33% of participants, and the suggested endotoxin exposure limit was exceeded among 93% of the participants. In addition, the prevalence of symptoms and distribution of work duration in the study population were reasonably consistent with other published studies. Because exposures were robust and since statistically significant results were observed, even after adjusting for potential confounding, the possibility of false negative results seems unlikely. Nasal lavage provided for nonsubjective identification of individuals with nasal inflammation. However, this is a dynamic process and it is not possible to control for all explanatory factors in a workplace setting. These uncertainties therefore limit to some degree the interpretation or representativeness of the results.

Some inconsistencies between our results and the existing literature are also noteworthy. Despite previous reports of prevalent respiratory symptoms among agricultural workers (approximately 23–50%) (Linaker & Smedley, 2002), post-shift symptoms in this study were not changed among participants with increased dust, endotoxin, or 3-OHFA exposures. This may have been due to the lack of a completely unexposed comparison group or to a healthy worker effect. Further, some participants may have already developed tolerance to the acute effects of organic dusts, and exposure monitoring over a single work shift may not have captured the critical exposure interval (Broad et al., 2006; Dosman et al., 2006). A lack of symptoms among endotoxin-exposed workers was also noted by other investigators (Krajewski et al., 2004; Laitinen et al., 2001). For example, there was no association between total 3-OHFA and respiratory symptom prevalence among 77 workers in various settings including slaughterhouses, grain/vegetable storage, or the animal feed industry, although some symptoms were increased in response to specific 3-OHFA exposures, primarily C14:0

(Laitinen et al., 2001). In our study, certain inflammation markers (PMN, albumin, ECP) were elevated among those with headaches, phlegm, or cough, suggesting that measurement of nasal airway inflammation may be more sensitive to the acute effects of endotoxin than self-reported symptoms.

Personal exposure to inhalable dust was associated with an unexpected decrease in nasal lavage fluid PMN and MPO. This observation may have been influenced by the development of tolerance among participants, or may have been due to the presence of an unmeasured inhibitory substance in the sampled particulate matter. There were also some inconsistencies between results obtained using the endotoxin rFC assay or 3-OHFA measures normalized to the air volume sampled as opposed to the mass of dust collected. Peak exposure events were not captured by the TWA measures, but may have played a role in these inconsistencies. Further, it may be that endotoxin or 3-OHFA within agricultural dust does not elicit a biological response until a threshold concentration is reached within the dust. Results in Table 2 indicate that endotoxin or 3-OHFA concentrations in dust (airborne endotoxin or 3-OHFA divided by airborne dust) were greatest in feedlot samples. Approximately 60–70% of participants in the upper quartile of exposure to endotoxin or 3-OHFA concentrations in dust were located in feedlots, and about 20% in this group worked in grain elevators. Although it was not possible to separately evaluate each type of facility due to sparse data, the results suggest that feedlots or other areas with elevated 3-OHFA concentrations in dust are more strongly associated with nasal inflammation. Variation in other unmeasured, pro-inflammatory substances (e.g., glucans, fungi, muramic acid, gram-positive bacteria, transitional metals, silica, diesel exhaust particulates, pesticides, antibiotics) in dust from the various work sites may have also contributed to the variable responses that were observed. Differences in the rank ordering of mean dust, endotoxin, and 3-OHFA levels among the facilities measured in this study support this possibility (Table 2). Note that corn farmers encountered the lowest mean endotoxin (rFC) levels but had some of the highest 3-OHFA exposures.

To evaluate the extent to which endotoxin tolerance may have played a role in this study, analyses were performed in which the relationship between personal endotoxin exposure and inflammation markers was evaluated among workers grouped according to questionnaire items potentially linked with chronic organic dust exposure (work duration, or time spent in grain elevators or on farms). Although there was a broad range of work duration among participants in this study (1 to 48 yr), it did not strongly modify responses associated with 3-OHFA exposure. However, participants with a shorter frequency or duration of farm work tended to have elevated levels of lavage fluid PMN, IL-8, albumin, and MPO in response to 3-OHFA exposures. These findings suggest that workers with less work experience on farms were more responsive to the acute effects of endotoxin on nasal inflammation compared to those with more farm experience. Extended farm work exposures may therefore confer a degree of tolerance to endotoxin's effects, consistent with other studies (Broad et al., 2006; Von Essen & Romberger, 2003). Alternatively, there may have been self-selection or a healthy worker effect in which more experienced workers represented a more phenotypically resistant population. A similar tendency was noted for albumin concentrations among those with less work experience in grain elevators or corn storage areas, whereas other measures of nasal inflammation in response to 3-OHFA were either enhanced (PMN, MPO) or unaffected (IL-8, ECP) by this type of work. The findings highlight the complex nature of airway inflammation in response to multiple reactive agents among individuals with varying degrees of susceptibility in different agricultural settings. Results from this analysis suggest that the development of endotoxin tolerance may vary due to differences in the responsiveness of inflammatory mediators to agricultural particulates, to variations in work duration, or to differences in the composition of agricultural dusts among work sites.

CONCLUSIONS

In summary, this study quantified personal work-shift exposures to inhalable dust, endotoxin, and its 3-OHFA constituents among workers in several agricultural settings. Exposures were compared to biomarkers of inflammation in post-work-shift nasal lavage fluids. The cross-sectional nature of this study did not allow for causal associations between 3-OHFA exposures and adverse respiratory outcomes to be evaluated. Nonetheless, elevated concentrations of 3-OHFA in workplace ambient particulate matter were associated with robust, statistically significant increases in nasal airway inflammation, indicating that these agents may serve as important indicators of the biological potency of airborne agricultural dust. This was the first study to evaluate the relationship between endotoxin's 3-OHFA constituents in agricultural dust and nasal airway inflammation. Additional research is needed to characterize the extent to which these agents may impair pulmonary function or contribute to respiratory disease among agricultural workers.

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